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Original Research Article

Effects of Soya Beans and Brown Beans on the Neurobehaviour of Cassava-induced Konzo Disease Rat Model

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Abstract

Background: Konzo disease is a neurological condition that affects the upper and lower motor neurons. This disease is prevalent in females and youngsters. The effects of soya beans and brown beans on neurobehavioural deficit in Cassava-induced konzo disease rat model were investigated in the present study. ***Materials and Methods***: 30 male wistar rats weighing 200-250g were assigned to Group 1 (Control, n=5). They were fed on animal pellet, whereas Group 2 (Soya and Brown Beans, n=5) was provided Soya and Brown Beans. Bitter cassava flour was provided to Group 3 (cassava induced Konzo, n=15). Protein (Soya and Brown Beans) and bitter cassava flour were supplied to Group 4 (protein treatment group, n=5). Neurobehavioural paradigms (Forelimb grip strength and gait test) were carried out to assess the effect of cyanogenic bitter cassava on motor coordination. ***Result and Discussion*:** Forelimb grip strength test showed significant decrease in the grip strength in Cassava group compared to Control and Protein(Soya and Brown Beans) group ($p<0.01$). It was observed that there was significant increase in grip strength test in the Cassava+ Protein (Soya beans and Brown Beans) when compared to Cassava group ($p<0.05$). Gait test showed significant decrease in the stance, stride and sway length of Cassava group when compared to the Control and Protein (Soya and Brown Beans) group ($p<0.01$). However, Cassava+ Protein group had a significant increase in stance, stride and sway length when compare to Cassava group ($p<0.05$). ***Conclusions*:** This study has provided a reference data on the ameliorative effects of Soya beans and Brown Beans on the neurobehavior of Cassava induced Konzo disease rat model. The ameliorative effects may be as a result of the presence of flavonoids and tannins in the brown beans and soya beans. This study will be useful to the Anatomists and Neuroscientist.

Keywords: Bitter cassava, Soya beans, Brown beans, Cyanogenic, Konzo.

INTRODUCTION

The rapid onset of a non-progressive, single, and symmetric spastic paraparesis characterizes konzo. A spastic gait is the primary symptom of the condition [1]. Prevalence is higher in females and younger people compared to adult males. While consuming toxic cassava products in large quantities for several weeks beforehand seems to be necessary for konzo development. One striking aspect of the illness is that it manifests suddenly within days or even hours, strongly indicating that a specific set of neurons is degenerating in a short period of time. Konzo is caused by unknown causal agent(s) and pathogenic processes. Insufficiently processed cassava flour contains significant amounts of cyanide, acetone cyanohydrin, and linamarin. Exposure to cyanide and linamarin is known to trigger the acute phases of konzo [2]. But konzo is a chronic illness with quite distinct symptoms from acute cassava poisoning, and it is invariably connected to a habit of long-term cassava eating [3]. In addition, none of the specific konzo traits are consistent with any of the recognized signs of acute or long-term cyanide exposure in people or animals, such as convulsions and delayed Parkinsonism [4]. The first is that the cyanide metabolite konzo may be caused by, including cyanate, thiocyanate, and 2-iminothiazolidine-4-carboxylic acid [5]. The second is that insufficient sulphur amino acid ingestion could impair rhodanese's ability to convert cyanide to thiocyanate [6]. As a result of the inappropriate eating of bitter cassava, there is an animal model of konzo [1]. Inspite of the fact that, Konzo disease is a challenge in Africa, there is scarcity of literatures on the effects of Brown Beans and Soya Beans on the neurobehavior of Cassava-induced Konzo disease rat model. This is the driving force behind this research. This study seeks to determine the effects of

soya beans and brown beans on the neurobehaviour of Cassava-induced Konzo rat model.

MATERIALS AND METHODS

Plant Collection and Identification

The roots of the bitter cassava, soya beans and brown beans were obtained from the Department of Crop and Soil Sciences, Faculty of Agriculture, University of Port Harcourt.

Bitter Cassava Root Processing

The farm's fresh cassava roots were uprooted. The cortex was scraped using a cutter shortly after harvesting to reveal the whitish interior layer. The cassava roots were then chopped into tiny pieces, similar to pommes frites, and sun-dried for three days. Cassava chow was made from pommes frites cassava that were grounded into powdered form using a grounding machine and fed to the animals used for the experiment [1].

Processing of Protein Food Supplement

For this study, a protein dietary supplement consisting of a mixture of soya bean and brown bean was served. Brown beans and soya beans were ground together in a grounding machine into a powdered form that was offered as protein diet nutrient to the "protein (soya beans and brown beans) group" and " cassava + protein (soya beans and brown beans) treatment group" animals, respectively

Experimental Animals and Maintenance

Thirty (30) Male Wistar rats weighed range of 200 and 250 grams were gotten from the Department of Pharmacology's animal home for this study. All of the Wistar rats were kept in their own regular polypropylene cages. The Wistar rats were acclimatized in the duration of three (3) weeks; maintained in laboratory standard conditions (12-hour light/dark cycles) and had free rein to an animal pellet feed including water. Animals were maintained under a controlled room temperature. The cage was designed, and crass saw dust was sprayed on the floor of the cage that act like protective cushion for the animals. There was replacing of crass saw dust every day to avoid accumulation of waste droppings so as to maintain adequate hygiene. The experimental animals were divided into four groups:

Group 1 (the negative control group, n=5) was given water and pelleted animal feed, whereas Group 2 (protein group, n=5) was given water and a protein-rich meal (soya beans+brown beans).

Group 3 (the cassava-induced Konzo group, n=15) received bitter cassava flour.

Group 4 (protein treatment group) n=5. The rehabilitation group (group 4) was entirely weaned off the bitter cassava after three weeks and replaced with soya beans and brown beans for the final two weeks after the Konzo illness induction period.

Oral intake was the method of nourishment. The weights of the animals were recorded on a weekly basis using an electric weighing scale. Physical symptoms and clinical indications were thoroughly monitored in the animals. The study took place over a five-week period.

Phytochemical Screening

The qualitative screening of soya beans and brown beans for phytochemical constituents to determine the presence of Flavonoids, Isoflavones and Tannin were done.

Behavioural Test: Forelimb grip test

We examined motor function and impairments in the rats with forelimb grip test. In this study, the traditional grip wire was used. The rat immediately grabbed the wire grid after placing the rat's tail horizontally on it, inverting the horizontal wire bar downward and suspending it from the ground. The maximum time the rat remained in the wire grid before removing its forepaws was timed using a stop watch while being observed by another inspector. To give the animals a chance to rest, this procedure was carried out three times at 10-minute intervals, and the average resting duration was noted.

Gait Test

Gait test is used to access motor coordination in rat models. Videotape recordings of experimental rats were examined for several gait metrics, including stride length (cm), stance, and sway phase (seconds). The length of a stride is the distance covered by one foot during a gait cycle, measured in centimeters. All animals' stride lengths (walk lengths) were measured using a metric rule. Rats' forepaws were dipped in ink and allowed to run on a strip of paper to measure the length of each stride. The forelimb stride lengths were manually measured as the mean distance between two forepaw prints. For the statistical analysis, the three middle stride lengths from each run were chosen. Because of differences in velocity, the paw prints left at the start and finish of each run were not included. The distance between the ipsilatera1 toes touching the ground was used to calculate stance distance. The ipsilateral toe's distance from the ground was what determines the Sway length, on the other hand [8].

Ethical Consideration

According to the University of Port Harcourt animal care and Research ethical committee, the ethical use of animals in research was approved. The ethical Clearance number is UPH/CEREMAD/REC/MM87/037.

Statistical Analysis

The data was analyzed using Graph Pad Prism (version 8.0) and Microsoft Excel (2016 edition). Values were presented in descriptive statistics as Mean ± SEM. A Tukey post-hoc multiple comparison test was

used after a one-way analysis of variance (ANOVA) to evaluate whether there was a significant difference between the groups. A result of P<0.05 was considered significant since the confidence interval was set at 95%.

RESULTS

The phytochemical screening of the soya bean and brown beans is shown in table 1 and 2. It showed positive results for flavonoids, Isoflavones and tannins.

Table 1: Phytochemical Analysis of Soya Beans

Sample	Qualitative screening
Flavonoids	++
Isoflavones	+++
Tannin	++

Table 2: Phytochemical Analysis of Brown Beans

Sample	Qualitative screening
Flavonoids	+++
Isoflavones	++
Tannin	+++

Effect of Cassava and Protein Diets on Motor Behaviour

Paw Print Test

(a) Stance Length

The result of stance length for the control group and the treatment groups are shown in figure 1. Using a one –way analysis of variance the result showed a significant decrease in the stance length (cm) of Cassava group [1.725±0.1887] compared to Control [4.950±0.7708; p<.01] and Protein [4.875±0.4553; p<.01] groups but not Cassava + Protein [3.252±0.3172] group (Figure 1).

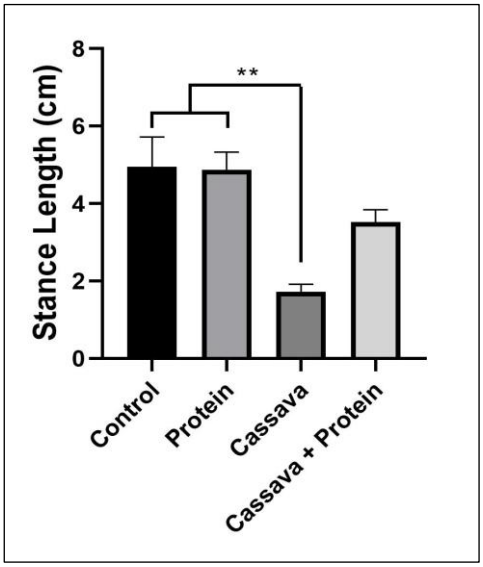

Figure 1: Shows the stance length of control and treated groups. Each column represents mean ± S.E.M. N=5/group. Data was examined using one-way analysis of variance followed by Tukey's posthoc-test. **p < 0.01

i. Stride Length

The result of stride length for the control group and the treatment groups are shown in Figure 2. Using a one –way analysis of variance the result showed significant decrease in the stride length (cm) of Cassava group [1.675±0.1787] compared to Control [4.820±0.7508; p<.01] and Protein [4.670±0.4053; p<.01] groups but not Cassava + Protein [3.252±0.3172] group (Figure 2).

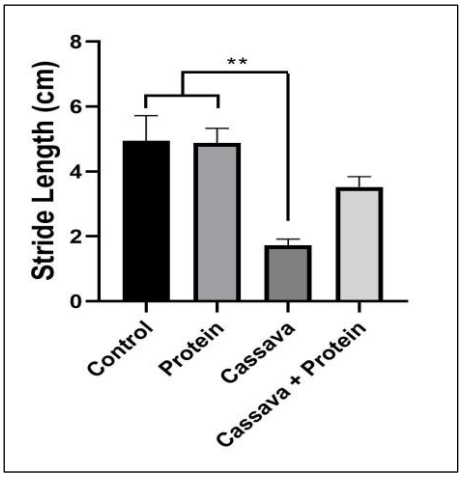

Figure 2: Stride length of control and treated groups. Each column represents mean ± S.E.M. N=5/group. Data was examined using one-way analysis of variance followed by Tukey's post-test. **p < 0.01

(C) Sway Length

The result of sway length for the control group and the treatment groups are shown in figure 3. Using a one –way analysis of variance the result showed significant decrease in the sway length (cm) of Cassava group [1.710±0.1067] compared to Control [4.756±0.6708; p<.01] and Protein [4.595±0.4223; p<.01] groups but not Cassava + Protein [3.201±0.3272] group (Figure 3).

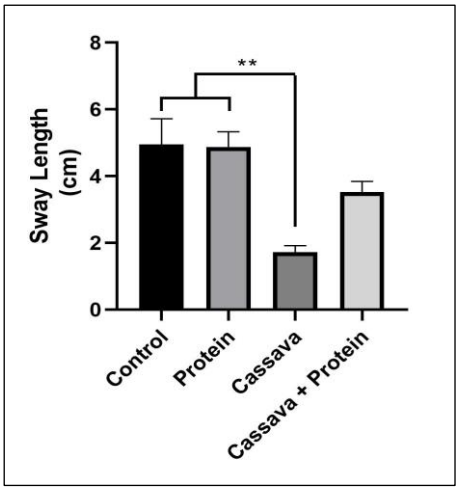

Figure 3: Sway length of control and treated groups. Each column represents mean ± S.E.M. N=5/group. Data was examined using one-way analysis of variance followed by Tukey's posthec-test. **p < .01

Grip Strength Test

The result of Grip Strength Test for the control group and the treatment groups are shown in figure 4. Using a one –way analysis of variance the result showed significant decrease in the grip strength in Cassava group [81.83±3.754] compared to Control [160.8±12.07; p<0.001], Protein [166.8±16.77; p<0.001], and Cassava + Protein [126.0±5.373; p<.05] groups (Figure 4).

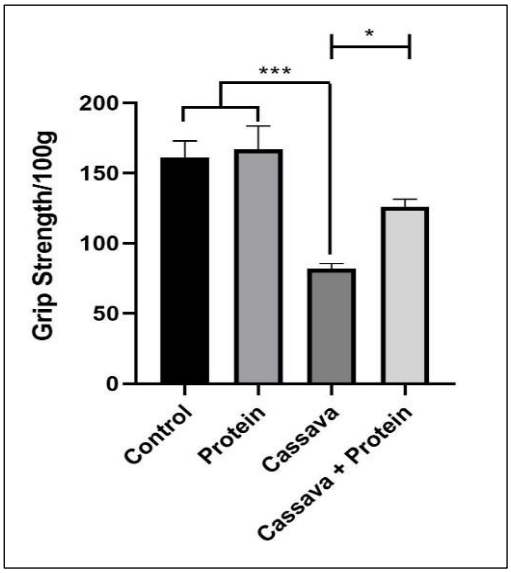

Figure 4: Grip Strength Test for the control and treated groups. Each column represents mean ± S.E.M. N=5/group. Data was examined using one-way analysis of variance followed by Tukey's posthoc-test. *p < 0.05, ***p <0.001

DISCUSSION

Clinical spastic paresis is a defining feature of the neurological condition konzo, which affects just certain upper motor neurons [1]. The typical warning signs of prolonged exposure to cyanide from improperly processed cassava roots include growth retardation, weight loss, and neurological diseases brought on by tissue damage in the CNS (central nervous system). Soya beans and brown beans contents (Flavonoid and Isoflavones), which are found in proteins, aid in the body's metabolism and excretion of cyanide [9]. To assess the neurolysin impact on the primary motor neurons and skeletal muscle functioning and how it can be mitigated with a protein-based diet. The present study replicated Konzo disease and its symptoms in an animal laboratory model.

This study employed two behavioral paradigms; forelimb grip test and gait tests (paw print test) to examine the motor implications of cyanogenic cassava diet. The gait tests were used to access locomotion coordination and grip test for overall muscle strength. The results showed significant reduction in the stride, sway, and stance length parameter of the gait test in the cassava fed rats (Figure 1–3). In a prior study, young rats fed a diet including cassava for a year displayed aberrant motor behavior [10]. Similarly, during the forelimb grip test, grip strength was significantly reduced. All these are indicative of locomotor abnormalities and overall motor deficits following prolonged cassava exposure (Figure 4). Other research have revealed that the harmful effect of cyanide, which is formed from the linamarin content in cassava juice during their biodegradation, is probably connected to poor motor coordination [11]. This concurs to the findings of [12] who explained that the effect of cassava juice also affects motor impairment. Interestingly, the motor functions in the present study may have been rescued due to the presence of Flavonoid and Isoflavones contents in soya beans and brown beans (protein diet supplements). This finding is in correlation with studies by [13, 14] which on the other hand was ameliorated by flavonoids content in Ginkogo biloba. Soya beans Isoflavones, prevents neuronal injury and cognitive decline [14].

CONCLUSION

This study has shown that soya beans and brown beans supplement has neuroprotective effect on the neurobehavior of Cassava-induced Konzo disease in Wistar rats. The data in this study, will be very useful to the Anatomists, konzo researchers and Neuroscientist.

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